Changes of the Electrical Excitability of Amphibian Muscle Fibre, and Discussion of A. V. Hill's Excitation Formula.

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Our present paper deals with the investigations to ascertain the influences of K and Ca chlorides, curare, and denervation, on the electrical excitability of muscle fibres, expressed in the curve relating to the duration and liminal strength of the stimulating constant current.

The whole of the experiments were carried out by means of the capillary pore electrode with the same stimulating arrangements as described in our previous paper (1). As the material of experiments we used exclusively M. sartorius of the frog, stimulating the extreme pelvic non-neural region on the ventral surface of the muscle.

To study the influences of various salts on the excitability we adopted two different methods, that is to say (1) to use Ringer's solution of various ionic concentrations, and to compare the results with those obtained by normal Ringer's solution; (2) to determine the excitability in bathing fluids of incomplete composition lacking either Ca or K chloride, and then to observe the changes produced by the increase of K or Ca chloride respectively, or by the addition of the missing salt, keeping other conditions as equal as possible during the whole series of experiments.

For the convenience of these experiments, and, at the same time, to prevent the change of osmotic pressure of the solution by adding salts, we prepared equimolecular stock solutions of 0·11 mol Na, K and Ca chlorides. As the normal Ringer's solution we employed the following composition:—

	Stock solutions in c.c.	Per cent. of salts.
NaCl	1,000	0·64
KCl	15	0·012
$CaCl_2$	20	0·024

The characteristic of a strength-duration curve may be expressed in terms of three quantities, that is, the minimum value of the liminal current-strength (λ), the minimum duration at which the current-strength is $\lambda (t_\lambda)$, and the current-duration at which λ is doubled ($t_{2\lambda}$). According to Lucas and Hill (2), (3), λ has no influence on the shape of the curve, therefore t_λ and $t_{2\lambda}$ are to be employed for the comparison of the excitatory process under various conditions, as any change in the shape of the curve refers to a change in the factors affecting directly the ease of concentration of ions, *i.e.*, the ease of production of the local excitatory disturbance.

On the normal strength-duration curve of frog's sartorius in Ringer's solution, we have found the following values at 16°–18° C.:—

$$\lambda = 1{\cdot}0\text{—}1{\cdot}3 \text{ microamperes},$$
$$t_\lambda = 0{\cdot}0025 \text{ sec.},$$
$$t_{2\lambda} = 0{\cdot}0003\text{—}0{\cdot}0004 \text{ sec.}$$

Influence of Ca *Ions.*

As shown in the following examples (fig. 1 and Table I), λ, t_λ, and $t_{2\lambda}$ increase with the quantity of Ca chloride in the solution (fig. 1).

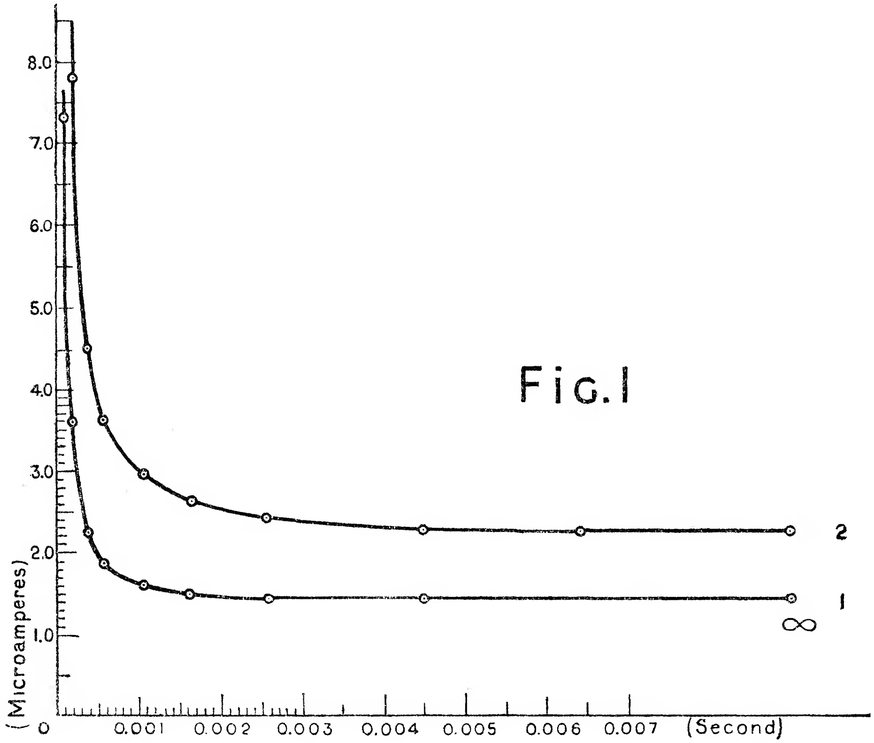

Fig. 1.—Influence of Ca chloride on the strength-duration curve.

(1) Bathing fluid: NaCl 150 c.c. (0·63 per cent.), $CaCl_2$ 3 c.c. (0·024 per cent.), 4 hours after excision, at 17·9° C.
$\lambda = 1{\cdot}438$ microamperes, $t_\lambda = 0{\cdot}0045$ sec., $t_{2\lambda} = 0{\cdot}00025$ sec.

(2) 6 c.c. $CaCl_2$ added to the bathing fluid, and the experiment made, after an hour, at 18·3° C. $\lambda = 2{\cdot}29$ microamperes, $t_\lambda = 0{\cdot}0064$ sec., $t_{2\lambda} = 0{\cdot}00035$ sec.

Table I.

(1) Bathing Fluid: NaCl 150 c.c., $CaCl_2$ 0·5 c.c., KCl 2·0 c.c., 4 hours after excision. Temperature 17·2° C.

(2) 6 c.c. $CaCl_2$ added and the experiment made after 1½ hours at the same temperature.

t (1/1000 sec.).	i_1 (microamperes).	i_2.
Inf.	0·477	0·543
10·22	0·477	0·543
8·31	0·477	0·546
6·40	0·477	0·558
4·49	0·477	0·573
3·54	0·477	—
2·57	0·480	0·591
2·19	0·486	—
1·62	0·504	0·621
1·05	0·543	0·681
0·57	0·681	0·871
0·38	0·879	1·113
0·19	1·607	1·986
0·10	4·691	4·855

	λ (microampere).	t_λ (sec.).	$t_{2\lambda}$ (sec.).
(1)	0·477	0·0035	0·00035
(2)	0·543	0·0102	0·0004

Influence of K *Ions.*

By increasing the quantity of KCl to some extent, the excitability for the current of relatively long duration increases, while the muscle fibre becomes less excitable towards the current of short duration; in short, the result shows the decrease of λ and increase of $t_{2\lambda}$, therefore the intersection of two curves occurs (fig. 2). Lucas and Mines (4) have obtained a similar crossing of curves in their experiments on the influence of temperature on the excitability of muscles and nerves, and concluded that the known conditions which led to the simultaneous appearance of the greater excitability towards long currents, and the rapid increase of liminal current-strength with decrease of current-duration, might be summed up simply as a slowing of the excitatory process.

Table II.

(1) Bathing Fluid: NaCl 150 c.c. (0·64 per cent.), $CaCl_2$ 0·5 c.c. (0·004 per cent.); 3 hours after excision, at 17·4° C.

(2) At the end of the first experiment 7 c.c. KCl (0·035 per cent.) was added, and after an hour the second series of experiments was carried out at the same temperature.

t.	i_1.	i_2.
Inf.	0·633	0·465
8·31	0·633	0·465
6·40	0·633	0·468
4·49	0·633	0·480
2·57	0·633	0·495
2·19	0·633	—
1·81	0·636	—
1·62	—	0·513
1·05	0·675	0·573
0·57	0·842	0·800
0·38	1·085	1·169
0·19	1·902	2·44
0·10	4·24	7·76

λ.	t_λ.	$t_{2\lambda}$.
(1) 0·633	0·00219	0·0003
(2) 0·465	0·00831	0·00055

Further increases of KCl in the solution leads to the decrease of excitability towards both long and short duration of stimulating currents, therefore crossing of the curves does not occur in this case (fig. 3).

Antagonism between Ca *and* K *Ions.*

(*a*) Supplying KCl to the bathing fluid of incomplete composition, which contains a fairly large quantity of $CaCl_2$, results in the increase of $t_{2\lambda}$ in spite of the decrease of λ and t_λ (fig. 4). But the further increase of KCl results in the increase of λ, t_λ and $t_{2\lambda}$, that is, the decrease of excitability.

(*b*) The diminished excitability of muscle fibre, which is immersed in the solution of incomplete composition such as NaCl + KCl, is restored to some extent by the addition of $CaCl_2$ (fig. 5).

In the solution with a higher concentration of KCl, say about 0·06 per cent., the excitability, and also the contractility of muscle fibres, were so diminished that a current of long duration and fairly high potential difference was necessary to produce weak and slow twitches, which, not being localised in the single fibre directly under the capillary pore of the electrode, were observed spreading over the whole area of the microscopic field. Even in this case we succeeded in restoring the excitability and contractility by adding a sufficient amount of $CaCl_2$. The following experiment shows this fact.

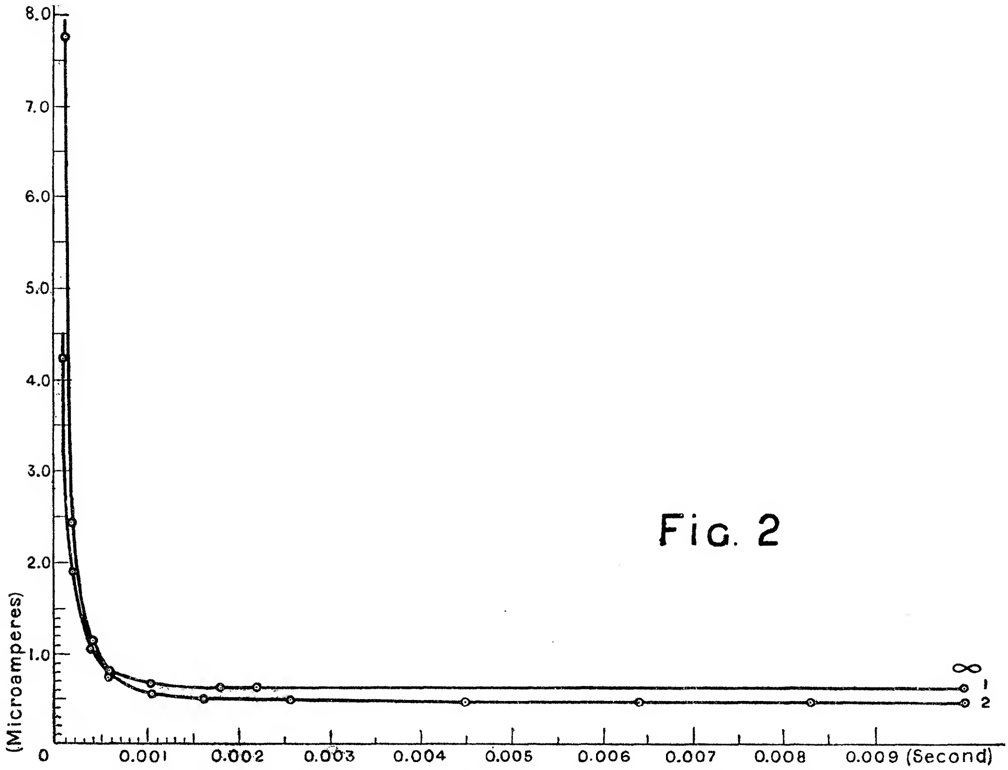

Fig. 2.—Influence of KCl, the crossing of two curves. Experimental data are given in Table II.

The dissected sartorius was immersed in the solution, which contained 150 c.c. of NaCl and 12 c.c. of KCl (0·06 per cent.). After 3 hours we commenced the experiment at 17·7° C. With the current of 8·37 microamperes and 0·03 second duration, we could scarcely observe the extremely weak and slow movement of muscle fibres over the whole microscopic field, and it was impossible to investigate regularly the relation of liminal current-strength to its duration. Then we added 15 c.c. of $CaCl_2$ to the bathing fluid, and, after 2 hours, we resumed the experiment. This time the twitch of muscle fibre was quick and localised in the single fibre, and the restoration of both excitability and contractility was obtained to such an extent as $\lambda = 1{\cdot}18$, $t_\lambda = 0{\cdot}0064$ and $t_{2\lambda} = 0{\cdot}0007$.

Mines (5) has studied the spontaneous movement of amphibian skeletal muscle in saline solutions, and concluded that the influence of $CaCl_2$ and KCl on muscular excitability is as follows: " The addition of $CaCl_2$ to NaCl solution surrounding the muscle leads to an immediate diminution or

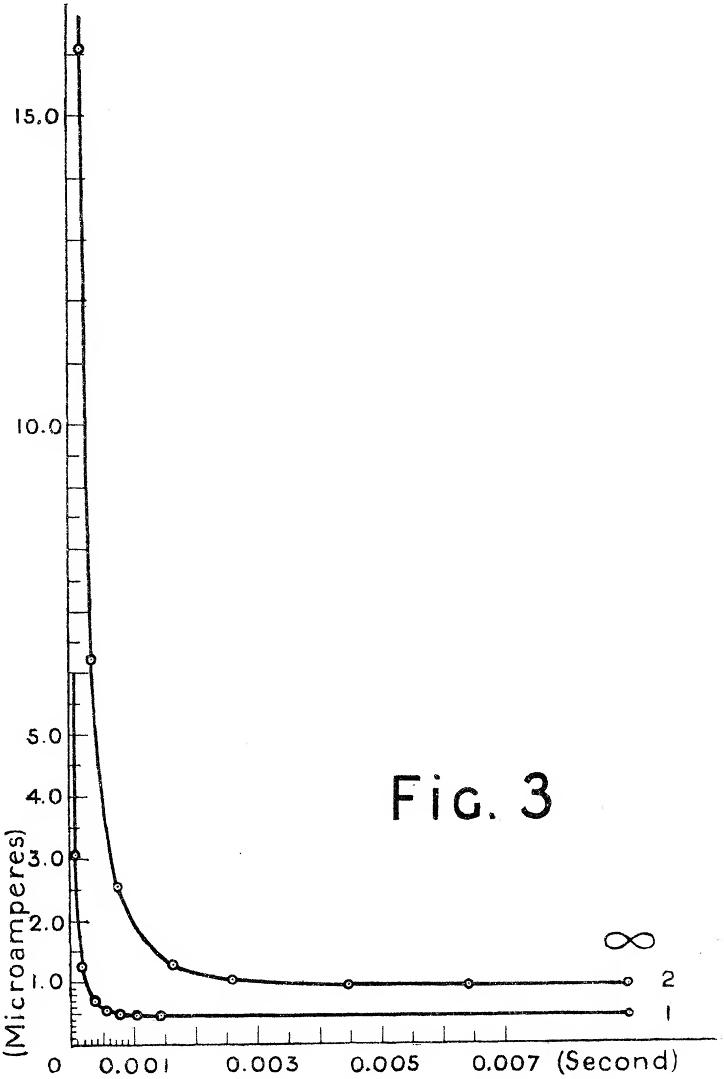

Fig. 3.—Influence of KCl.

(1) NaCl 150 c.c. and $CaCl_2$ 0·5 c.c.; 3½ hours after excision, at 18·3° C. $\lambda = 0{\cdot}452$, $t_\lambda = 0{\cdot}0016$ and $t_{2\lambda} = 0{\cdot}00027$.

(2) $\frac{N}{8}$ KCl 9 c.c. (0·046 per cent.) added and after 2 hours the experiment was made, at 18·4° C. $\lambda = 0{\cdot}947$, $t_\lambda = 0{\cdot}0064$ and $t_{2\lambda} = 0{\cdot}001$.

cessation of the movements, and a fall in the excitability of the non-neural region towards galvanic currents. KCl at first increases the movements, often causing a very rapid rhythm, and then stops them entirely: its effect on the excitability towards galvanic currents is a primary exaltation and a subsequent depression." Further, he described the interaction or antagonism between Ca and K ions.

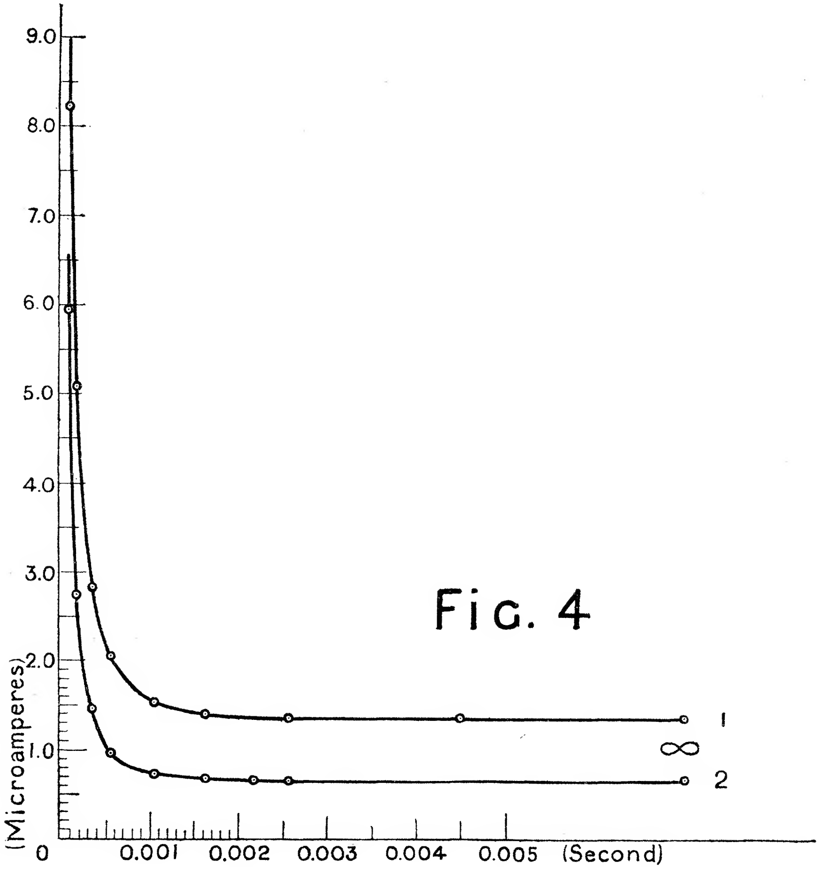

Fig. 4.—Interaction of $CaCl_2$ and KCl.
(1) NaCl 150 c.c. and $CaCl_2$ 6 c.c. (0·047 per cent.); 4½ hours after excision, at 18·1° C. $\lambda = 1{\cdot}353$, $t_\lambda = 0{\cdot}00449$, $t_{2\lambda} = 0{\cdot}00037$.
(2) KCl 7·5 c.c. (0·038 per cent.) added; 7 hours after excision, at 18·5° C. $\lambda = 0{\cdot}666$, $t_\lambda = 0{\cdot}00257$, $t_{2\lambda} = 0{\cdot}0004$.

Our experiments on a single muscle fibre confirm his conclusions on the action of $CaCl_2$ and KCl, as well as their antagonism.

As the changes of excitability which are produced by K or Ca ions can be restored more or less by the action of antagonistic ions, therefore, these changes may be reversible to some extent. A factor which makes us able to explain our experimental results is the alteration of boundary condition

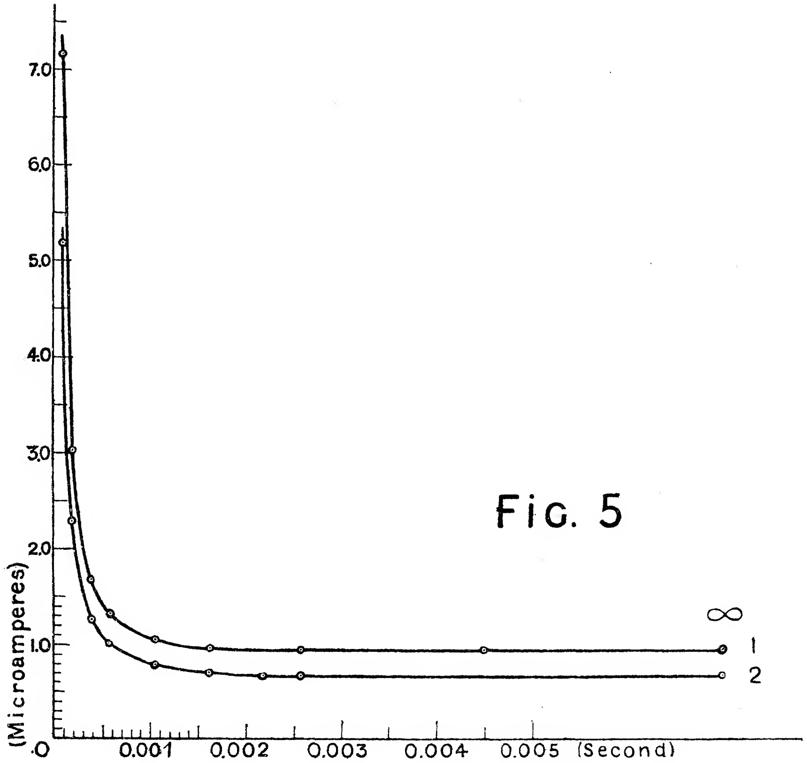

FIG. 5.—Interaction of KCl and $CaCl_2$.
(1) NaCl 150 c.c. and KCl 6 c.c. (0·032 per cent.) ; 3 hours after excision, at 17·9° C. $\lambda = 0·945$, $t_\lambda = 0·00449$ and $t_{2\lambda} = 0·00032$.
(2) $CaCl_2$ 7·5 c.c. (0·056 per cent.) added ; 6½ hours after excision, at 18·3° C. $\lambda = 0·669$, $t_\lambda = 0·00257$ and $t_{2\lambda} = 0·00035$.

of an impermeable membrane, especially the alteration of its permeability. Assuming that K ions make the membrane more permeable, while, on the contrary, Ca ions make it less permeable, we may suppose that, if the action of K ions is predominant, the stimulating currents will enter into an excitable element more easily, that is the positive factor for the excitability; but, on the other hand, some of the concentrated ions may possibly diffuse out more freely through the membrane—the negative factor. The decrease of λ and the increase of $t_{2\lambda}$ by a small dose of KCl may thus be explained as the net result of two opposing factors. The strong dose of KCl will increase the permeability to such an extent that, owing to the prevailing negative factor, the necessary amount of ionic concentration for the electrical excitation will hardly be obtained, while the electric currents will flow through many muscle fibres without any remarkable deformation, consequently causing a non-localised contraction of muscle fibres under strong stimuli. But even in such a condition of the membrane, the addition of $CaCl_2$ to the surrounding solution would make the membrane less permeable, in consequence of which the balance of the positive and negative factors may be re-established, and the excitability, and probably also the contractility, may be restored to some extent.

Influence of Curare.

According to Mines (5) the action of curare on the muscle element is to be accounted for by the influence of inorganic salts present in curare, possibly Ca salts. Lucas (6) has shown that the presence of 0·03 per cent. curare in the fluid soaking the muscle is sufficient to eliminate the excitability of β and γ substances, but not strong enough to influence the muscle element. On the whole, our experiments support those results. For example :—

Curare.	λ.	t_λ.	$t_{2\lambda}$.	Temperature.
per cent.				° C.
0·03	0·28	0·0022	0·0005	17
0·05	0·75	0·0064	0·0007	19·2

The former case is similar to the normal curve, while the latter shows much resemblance to the curve of Ca action.

Influence of Denervation.

The motor nerve of sartorius and other adductor muscles of the frog is R. descendens communis of R. profundus posterior of the sciatic nerve. Cutting the dorsal skin about 0·5 cm. at the proximal part of one leg along the sciatic nerve and finding R. descendens communis, we cut off about 1 cm. of it to prevent regeneration. The experiments were carried out 29 days after the operation. By pithing such a frog, we noticed that the operated leg was stretched in the abducted position, owing to the paralysis of adductor muscles. Throughout the dissection of sartorius muscle, not the slightest twitch was observed, even in the cutting of its motor nerve. Thus we ascertained the degeneration of the peripheral part of the divided nerve. The denerved muscle showed obviously an atrophic appearance and microscopically consisted of thinner fibres. Nevertheless, the excitability of muscle fibre seemed to be abnormally increased to such an extent that irregular and persistent fibrillary contractions were easily provoked by relatively slight mechanical stimuli during the procedure of dissection. After a soaking in Ringer's solution of relatively high $CaCl_2$ concentration, these peculiar contractions promptly stopped. The increased excitability and antagonistic action of Ca ions on it indicate the resemblance of the denerved muscle in this stadium to the normal muscle in the primary exaltation by a small quantity of KCl, and we expect further the final depression of excitability in the denerved muscle which may be influenced positively by Ca salts, as in the case of K action. If we adopt the membrane hypothesis on the action of salts, we may assume that the denervation makes the membrane more permeable and results in the increase of excitability. The relation of current-duration to the liminal strength suggests the possibility of such an assumption. For example, $\lambda = 0{\cdot}974$, $t_\lambda = 0{\cdot}0018$, and $t_{2\lambda} = 0{\cdot}0003$.

However, such a phenomenon should be regarded as the primary stadium of muscle degeneration, which causes finally the total disappearance of excitability. The increased galvanic and decreased Faradic excitability in the so-called "Entartungsreaktion" of muscle may be accounted for by such a condition of muscle fibres.

On the Excitation Formula.

In order to explain the nature of the changes of excitability under various conditions, we have to treat our experimental data quantitatively by means of the excitation formula. Lucas (2) has applied Hill's excitation formula (3), which was derived from the physical theory of excitation by an elaborate mathematical treatment, to the analysis of the observed differences between the excitatory process of different excitable tissues and to the analysis of the changes of excitability effected by various alterations of conditions, and he verified the validity of this formula, which is, in its simplest form,

$$i = \frac{\lambda}{1-\mu\theta^t}$$

where i is the smallest current which will excite, t is the duration of the current, and λ, μ, and θ are constants.

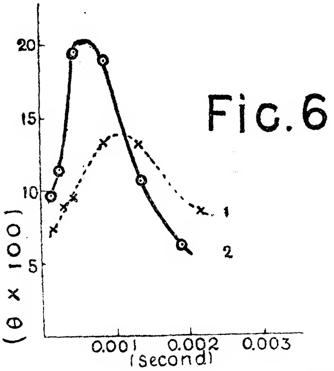

Fig. 6.—Variations of θ, calculated from two successive values of t and i.

On the forty-one cases of our experiments (including those in our previous paper) we calculated the value of θ, as described by Lucas; but, contrary to our expectation, we have found no case in which the value of θ could be regarded as constant. Moreover, the variation of θ (the difference of maximum and minimum values of calculated θ) seems to have some regularity; that is, in the case of denervation and KCl action it is rather smaller than in the case of $CaCl_2$ and curare. The experiment on the interaction between KCl and $CaCl_2$ reveals the possible relation between the variation of θ and action of salts. In the example shown in fig. 5, while the excitability of muscle fibre is obviously increased by the addition of $CaCl_2$, the variation of θ is more remarkable in the latter case (fig. 6).

Even from Lapicque's data which Lucas has described as the example of calculation, we cannot get a sufficiently constant value of θ, as is shown in the following Table:—

Table III.

t.	i.	θ.
1/3	175	0·373
2/3	115	
1·0	91	0·375
1·5	76	0·345
2·0	68	
2·5	64	0·298
3·0	61	0·139
Inf.	60	

From this point of view, we are inclined to suppose that θ is not a constant but a variable which may be influenced by the boundary condition of membrane.

In the course of mathematical treatment of the condition equations, Hill has assumed i = constant. But, as we have suggested in the previous paper, the deformation of constant current in the excitable tissue should be taken into consideration, therefore his assumption is invalid as well as unrealisable in the present arrangement of experiment. This is probably the reason, or at least one of the reasons, why the experimental data, however accurate the methods employed, do not agree with the theoretical deduction.

Now, as it is almost impossible to obtain a mathematically exact formula, satisfying all the possible conditions including the deformation of the stimulating current, we shall have to find, if possible, some empirical formula from the data obtained, to compare the effect of the electrical stimulation under various conditions and on the various excitable tissues. Our experimental curves relating to the current-duration and its liminal strength represent hyperbolæ of a higher order, which are generally expressed by

$$i - i_0 = \mathrm{A}(t - t_0)^{-m}.$$

But to find some regular relation between these constants m, A, t_0 and i_0, is the problem of our further investigation and calculation.

Summary.

(1) Increase of the quantity of Ca ions in the bathing fluid decreases the excitability, that is, the increase of λ (minimum value of the liminal current-strength), t_{λ} (minimum duration at which the current-strength is λ) and $t_{2\lambda}$ (current-duration at which λ is doubled) is obtained.

(2) The increase of K ions within a certain limit results in the decrease of λ, but on the other hand in the increase of $t_{2\lambda}$. In other words, for the excitation of muscle fibre, currents of less strength are required when currents of long duration are used, but the excitability towards currents of short duration is diminished. When a large quantity of KCl is applied, a weak twitch which is not localized in a single fibre is obtained by a current of relatively long duration. Even in such a case, by adding a proper quantity of $CaCl_2$ to the bathing fluid, both the excitability and contractility can be restored to some extent and the twitch is localised in a single fibre.

(3) The possible factor for the reversible influence of K and Ca ions may be found in the alteration of permeability of the membrane.

(4) The action of curare solution upon the muscle fibre resembles that of $CaCl_2$. The excitability of the muscle fibre remains unchanged when a small quantity of curare is added to the solution, but in large quantity it causes an alteration of the strength-duration curve in the same direction as do Ca ions. This may be due to the action of Ca ions contained in the curare solution.

(5) The alteration of the excitability of muscle fibre after excision of its motor nerve resembles that caused by the action of K ions.

(6) Calculating the value of θ in Hill's excitation formula, no case of our 41 experiments has been found in which θ has a constant value even approximately. There is a marked variation of θ when $CaCl_2$ or curare is given in large amounts, but it is somewhat reduced when KCl is added or denerved muscles are used. There seems to exist some relation between a decreased permeability of membrane and an increased variation of θ. The reason why Hill's formula is not applicable to the experimental results may be found in the invalid and unrealisable assumption that the stimulating constant current will not alter its shape as it enters an excitable element.

In conclusion we should like to take this opportunity of expressing our sincere thanks to Prof. H. Nagai and Prof. K. Hashida for their kind interest and advice.

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